APPENDIX A. VERIFICATION OF STATE CERTIFICATION

Please complete the following:

Laboratory Name: _____

Address: _____

City: _____ **State:** _____ **Zip:** _____

Contact Person: _____

Telephone: **( )** _____

Laboratory Type: **Utility:** ____ **Commercial:** ____ **State:** ____ **Other:** ____

Certification: The information requested in this section is necessary to verify the Drinking Water Laboratory certifications listed below. Please fill-out completely and supply all requested documentation.

ANALYTICAL METHODS PERFORMED	(Indicate Methods Performed with a ✓)	STATE(S) in Which Certified	CERTIFICATION	
			Type	Certification Date
TC-MF				
TC-MTF				
FC-MF				
FC-MTF				
EC + MUG				
ONPG - MUG				
NA + MUG				

Please attach a copy of your current letter(s) or certificate(s) of approval for conducting the above analyses and return to:

ICR Laboratory Coordinator
U.S. EPA, OGWDW
Technical Support Division
26 West Martin Luther King Drive
Cincinnati, Ohio 45268

APPENDIX B. APPLICATION FOR LABORATORY APPROVAL FOR THE INFORMATION COLLECTION RULE (ICR)

The U.S. Environmental Protection Agency (EPA) is proposing to require public water systems which serve 10,000 people or greater to generate and provide the Agency with specific monitoring data and other information characterizing their systems. Depending on the population served, systems which use surface water, or ground water under the direct influence of surface water, would be required to monitor their source water at the intake of each plant for two disease-causing protozoa, *Giardia* and *Cryptosporidium*, total coliforms and fecal coliforms or *Escherichia coli*. Systems which serve more than 100,000 people would be required to monitor their source water at the intake of each plant for the microorganisms indicated above, plus total culturable viruses. When pathogen levels equal or exceed one virus or protozoan per liter in the source water, systems would also be required to monitor their finished waters for these microorganisms.

Laboratories monitoring for protozoa and viruses would have to be approved by the U.S. EPA. The attached information describes the minimal requirements for approval to perform protozoan and/or virus analyses under the Information Collection Rule. Accepted applicants will also be required to demonstrate capabilities based on analyses of unknown samples and an on-site inspection of their facility.

Those interested in being approved must first demonstrate their qualifications by completing the attached application(s) and forwarding it (them) to:

ICR Laboratory Coordinator
U.S. Environmental Protection Agency
Office of Ground Water and Drinking Water
Technical Support Division
26 West Martin Luther King Drive
Cincinnati, Ohio 45268

Qualified applicants will be provided a copy of the **ICR Microbial Laboratory Manual** describing fully the approval requirements.

Since total coliform and fecal coliform/*E. coli* analyses proposed under the ICR are required under the Drinking Water Laboratory Certification Program, laboratory approval for these analyses is not required under the ICR if State certification can be verified (see **Appendix A**).

MINIMAL REQUIREMENTS FOR VIRUS LABORATORIES

Background Information:

For ICR approval, the virus analytical laboratories must have suitable facilities, equipment, instrumentation, and an ongoing quality assurance (QA) program. Analysts must be experienced in viral analyses and meet performance evaluation criteria. As laboratories are approved, the U.S. EPA will provide an updated list of those laboratories with Agency approved analysts to the public water systems that serve a population of 100,000 or more.

Analytical Methods:

The proposed virus protocol was published in the **Federal Register**, Vol. 59, No. 28, February 10, 1994, 40 CFR Part 141 **Monitoring Requirements For Public Drinking Water Supplies;** Proposed Rule; pp. 6430-6444. The final draft method will be provided to those applicants that meet the minimal requirements set forth in this document. The final method will be available at the time the ICR is promulgated.

Sample Collection:

Each analytical laboratory will be responsible for procuring, assembling, sterilizing, and transporting the sample collection apparatus to the water system. Systems will be advised on proper collection techniques by the analytical laboratory in accordance with the procedures in the ICR virus protocol. A virus sampling video will be available to the system to reinforce instructions received from the analytical laboratory.

Approval of an Analytical Laboratory:

The minimal requirements for personnel (education; training or equivalent experience), facilities, equipment and instrumentation, QA/quality control (QC), etc. listed below must be met and documented in the application before the laboratory and analysts will be judged qualified to be considered for approval. If the above criteria are met, ICR approval to perform analyses will require: 1) successful performance on QC samples, as defined in the virus protocol, 2) satisfactory analyses on unknown performance evaluation (PE) samples, and 3) an on-site evaluation of the laboratory and the analyst(s).

QC Samples/Cell Line:

EPA will provide QC samples containing known virus concentrations to laboratories meeting minimal requirements. These samples are to be used initially and periodically thereafter to demonstrate the analyst(s)' ability to process and analyze samples correctly. Buffalo green monkey (BGM) cells will be provided to establish uniform cell cultures in all laboratories.

Minimal Requirements:

1. **Personnel:**

Principal Analyst/Supervisor: To be qualified for approval, a laboratory must have a principal analyst who may also serve as a supervisor if an additional analyst(s) is to be involved. The principal analyst/supervisor oversees or performs the entire analyses and carries out QC performance checks on technicians and/or other analyst(s). This person must be an experienced microbiologist with at least a B.A./B.S. degree in microbiology or a closely related field and a minimum of three years continuous bench experience in cell culture propagation, processing of virus samples, and animal virus analyses. This analyst must have analyzed a PE sample set using the ICR virus method and results must fall within acceptance limits. Also, the principal analyst must demonstrate acceptable performance during an on-site evaluation by U.S. EPA personnel.

Analyst: This person(s) performs at the bench level under the supervision of a principal analyst and can be involved in all aspects of analysis, including preparation of sampling equipment, filter extraction, sample processing, cell culture, virus assay, and data handling. The analyst must have two years of college lecture and laboratory course work in microbiology or a closely related field. The analyst must have at least six months bench experience in cell culture and animal virus analyses, including three months experience in filter extraction of virus samples and sample processing. Six months of additional bench experience in the above areas may be substituted for the two years of college. Each analyst must have analyzed a PE sample set using the ICR virus method and results must fall within acceptance limits. The analyst must also demonstrate acceptable performance during an on-site evaluation.

Technician: This person extracts filters and processes the samples under the supervision of an analyst, but does not perform cell culture work, virus detection or enumeration. The technician must have at least three months experience in filter extraction and processing of virus samples.

2. **Laboratory Facilities:** Laboratories must have an air system regulated for temperature, humidity and air cleanliness. Laboratories should be maintained under negative air pressure to protect against accidental release of viral pathogens and should be equipped with ultraviolet lights for decontamination of rooms during periods when personnel are absent. Laboratories should maintain separate rooms for preparing cell cultures and processing virus samples. However, in the absence of separate rooms, laminar flow hoods must be used for cell culture preparation to prevent contamination. Freezers, incubators, and other large instruments should be in rooms where they can be accessed without disturbing ongoing laboratory efforts. The area provided for preparation and sterilization of media, glassware, and equipment should be separate from other laboratory work areas, but close enough for convenience. Visitors and through traffic must be minimized in work areas. ICR samples will be archived for future

testing by polymerase chain reaction (PCR) methods which are sensitive to contamination. Therefore, rooms for processing and assaying ICR samples must not have been used for analyzing PCR products. For ICR studies, the minimal area recommended for each worker is six to ten linear feet of usable bench space per analyst, exclusive of areas requiring specialized equipment or used for preparatory and supportive activities. Bench tops should be stainless steel, epoxy plastic, or other smooth impervious material that is inert and corrosion-resistant. Laboratory lighting should be even, screened to reduce glare, and provide about 100 foot-candles of light intensity on working surfaces.

High standards of cleanliness must be maintained in work areas. Laboratory bench surface cleanliness and laboratory air quality must be monitored. The laboratory must have a pest control program that includes preventive measures such as general cleanliness and prompt disposal of waste materials. The laboratory must be in compliance with all applicable judicial ordinances and laws for the managing and disposal of pathogenic agents.

3. **Laboratory Equipment And Instrumentation:** The laboratory must be equipped on-site with the instrumentation and equipment needed to perform the virus sample collection, extraction, concentration and assay as set forth in the ICR virus protocol. Included are incubators, water baths, hot air sterilizing ovens, autoclaves, refrigerators with -20°C freezer compartment, -70°C deep freezers, reagent grade water supply, balances, pH meter, centrifuges, temperature recording devices, and both upright and inverted microscopes. Laminar flow hoods and UV lights are strongly recommended as added equipment within the analytical laboratory.

4. **Safety:** Laboratory must meet Biosafety Level 2 Criteria as described in **Biosafety in Microbiological and Biomedical Laboratories,** 3rd Ed., HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, May, 1993. **Immunocompromised individuals must not work in or be admitted to this area**.

5. **QA/QC Procedures:** A formal QA document must be prepared and should follow the guidelines for a laboratory QA Plan, p. 7 in the **Manual for the Certification of Laboratories Analyzing Drinking Water**, 1990, U. S. Environmental Protection Agency Publication No. EPA/570/9-90/008, 3rd Ed., Washington, D.C., and Section II of this Manual. Laboratories must have a written QA program that applies practices necessary to minimize errors in laboratory operations that are attributable to personnel, equipment, supplies, processing procedures, or analytical methods. These include records of routine monitoring of equipment and instrumentation performance. Records of QC checks must be available to the U.S. EPA for inspection. The procedures for preparation of reagents and cell cultures and performance of the method must be followed exactly as written in the U.S. EPA ICR virus method. Reagents must be stored no longer than the designated shelf life.

6. **Record-Keeping And Data Reporting:** A record system must be in use for tracking the samples from sample collection through log-in, analyses and data reporting.

INFORMATION COLLECTION RULE

APPLICATION FOR APPROVAL OF VIRUS LABORATORIES AND ANALYSTS [1]

Laboratory: _____

Address: _____

City: _____ **State:** _____ **Zip:** _____

Contact Person: _____

Title: _____

Telephone: ( ) _____ **Fax:** ( ) _____

Type of Laboratory: **Commercial** ____ **Utility** ____ **State** ____ **Academic** ____

Other (describe) _____

Principal Customers: **Environmental** ____ **Clinical** ____ **Other** _____

Type of Virus analyses: **Human** ____ **Animal** ____ **Bacterial** ____

Other (describe) _____

PERSONNEL QUALIFICATIONS

Name, education, virus analysis experience and field in which acquired (water, waste-water, soils/sludge, shellfish, clinical, etc.)

Principal Analyst/Supervisor: _____

Education [University/Degree(s)]: _____

Experience: _____

_____

_____

_____

[1]Where additional pages are required, clearly mark them using the same headings as in this application form.

Analyst #1: _____

Education: _____

Experience: _____

Analyst #2: _____

Education: _____

Experience: _____

Analyst #3: _____

Education: _____

Experience: _____

Technician #1: _____

Education: _____

Experience: _____

Technician #2: _____

Education: _____

Experience: _____

Technician #3: _____

Education: _____

Experience: _____

ON-SITE LABORATORY EQUIPMENT AND INSTRUMENTATION

ITEM	On[a] Order	Number	TYPE/MODEL
Reagent Water System			
Sterilizing Oven			
Incubator			
Centrifuge			
pH Meter			
Temperature Recorder			
Inverted Microscope			
Upright Microscope			
Autoclave			
-70°C Freezer			
Refrigerator			
Analytical Balance			
UV Light System			
Water Bath			
Other(s) (describe)			
[a]Place a "✓" in the "On Order" column next to items that are on order.			

CURRENT LABORATORY PROGRAMS

Virus Method(s) (processing, assay)	Number of Analyses Per Year Per Method and Virus Groups Analyzed

Sample Types (Matrices Tested): _____

_____

_____

Cell Culture (Mammalian): _____

_____

_____

Documented Laboratory QA Plan: **Yes____** **No____**

Laboratory is in compliance with state and local ordinances and laws for handling and disposal of pathogenic agents:

Yes____ **No____**

Comments: _____

_____

_____

Estimated number of water samples that can be analyzed for virus/month using the method: _____

_____

_____

The above application information is complete and accurate to the best of my knowledge.

_____

Laboratory Manager or Designee

Submit Application to:

ICR Laboratory Coordinator
U. S. Environmental Protection Agency
Office of Ground Water and Drinking Water
Technical Support Division
26 West Martin Luther King Drive
Cincinnati, OH 45268

MINIMAL REQUIREMENTS FOR PROTOZOAN LABORATORIES

Background Information:

For ICR approval, the protozoan analytical laboratories must have suitable facilities, equipment, instrumentation, and an ongoing quality assurance (QA) program. Analysts must be experienced in protozoan analyses and meet performance evaluation criteria. As laboratories are approved, the U.S. EPA will provide an updated list of those laboratories with Agency approved analysts to the public water systems that serve a population of 10,000 or more.

Analytical Methods:

The proposed protozoan method was published in the **Federal Register**, Vol. 59, No. 28, February 10, 1994, 40 CFR Part 141 **Monitoring Requirements For Public Drinking Water Supplies;** Proposed Rule; pp. 6416-6429. The final draft method will be provided to those applicants that meet the minimum requirements set forth in this document. The final method will be available at the time the ICR is promulgated.

Sample Collection:

Analytical laboratories will be responsible for procuring, assembling, and transporting the sample collection apparatus to the water system. Systems will be advised on proper collection techniques by the analytical laboratory in accordance with the procedures described in the protozoan protocol. A sampling video will be available to the systems to reinforce instructions received from the analytical laboratory.

Approval of the Analytical Laboratory:

The minimal requirements for personnel (education; training or equivalent experience), facilities, instruments, QA/QC, etc. listed below must be met and documented in this application before laboratories and analyst(s) will be judged qualified to be considered for approval. If the above criteria are met, ICR approval to perform analyses also will require: 1) recovery of both *Giardia* cysts and *Cryptosporidium* oocysts from QC samples, 2) satisfactory analyses on unknown PE samples and 3) an on-site evaluation of the laboratory and the analyst(s).

QC Samples:

The U.S. EPA will provide QC samples containing known *Giardia* and *Cryptosporidium* concentrations to laboratories meeting minimal requirements. These samples are to be used initially and periodically thereafter to demonstrate the analyst(s)' ability to process and analyze samples correctly.

Minimal Requirements:

1. **Personnel:**

Principal Analyst/Supervisor: To be qualified for approval, a laboratory must have a principal analyst who may also serve as a supervisor if an additional analyst(s) is to be involved. The principal analyst/supervisor oversees or performs the entire analyses and carries out QC performance checks on technicians and/or other analysts. The principal analyst/supervisor must confirm all protozoan internal structures demonstrated at the microscope by subordinates. This person must be an experienced microbiologist with at least a B.A./B.S. degree in microbiology or a closely related field. The principal analyst also must have at least one year of continuous bench experience with immunofluorescent antibody (IFA) techniques and microscopic identification and have analyzed at least 100 water and/or wastewater samples for *Giardia* and/or *Cryptosporidium*. In addition, PE samples must be analyzed using the ICR protozoan method and results must fall within acceptance limits. The principal analyst/supervisor must also demonstrate acceptable performance during an on-site evaluation.

Analyst: This person(s) performs at the bench level under the supervision of a principal analyst/supervisor and is involved in all aspects of the analysis, including preparation of sampling equipment, filter extraction, sample processing, microscopic protozoan identification, and data handling. Recording presence or absence of morphological characteristics may be done by the analyst but must be confirmed by the principal analyst. The analyst must have two years of college lecture and laboratory course work in microbiology or a closely related field. The analyst also must have at least six months bench experience, must have at least three months experience with IFA techniques, and must have analyzed at least 50 water and/or wastewater samples for *Giardia* and/or *Cryptosporidium*. Six months of additional bench experience in the above areas may be substituted for two years of college. In addition, PE samples must be analyzed using the ICR protozoan method and results must fall within acceptance limits. The analyst must also demonstrate acceptable performance during an on-site evaluation.

Technician: This person extracts filters and processes the samples under the supervision of an analyst, but does not perform microscopic protozoan detection and identification. The technician must have at least three months experience in filter extraction and processing of protozoa samples.

Laboratory Facilities: The laboratory must have dedicated, well-lighted bench space commensurate with the number of samples to be analyzed. Six to ten feet of usable bench space are required per analyst, exclusive of areas requiring specialized equipment or used for preparatory and supportive activities. Bench tops should be stainless steel, epoxy plastic or other smooth impervious material that is corrosion-resistant. Laboratory lighting should be even, screened to reduce glare, and provide 100 foot-candles of light intensity on working

surfaces. Laboratory floor space must be sufficient for stationary equipment such as refrigerators and low-speed and large-capacity centrifuges. Facilities for washing and sterilization of laboratory glassware, plasticware and equipment must be present. A dedicated space that can be darkened must be available for the microscopic work. Laboratory areas should be kept free of clutter and equipment and supplies should be stored when not in use. It is strongly recommended that laboratories should be maintained under negative air pressure to protect against accidental release of pathogens and should be equipped with ultraviolet lights for decontamination of rooms during periods when personnel are absent. High standards of cleanliness must be maintained in work areas. The laboratory must have a pest control program that includes preventive measures such as general cleanliness and prompt disposal of waste materials. The laboratory must be in compliance with all applicable judicial ordinances and laws for management and disposal of pathogenic agents.

3. **Laboratory Equipment And Instrumentation:** The laboratory must be equipped on-site with a reagent water supply system, autoclave, refrigerator (4°C) with -20°C freezer compartment, pH meter, slide-warming tray or incubator (37 ± 3°C), balance (top loader or pan), membrane filtration equipment for epifluorescent staining, and hydrometer set. Specific requirements for the microscope include differential interference contrast (DIC) or Hoffman modulation optics (including 20X and 100X objectives). DIC or Hoffman modulation optics should have epifluorescence capability. The epifluorescence vertical illuminator should have either a 50 or 100 watt high-pressure mercury bulb with appropriate excitation and band-pass filters (exciter filter: 450-490 nm; dichroic beam-splitting mirror: 510 nm; barrier or suppression filter: 515-520 nm) for examining fluorescein isothiocyanate-labeled specimens.

4. **Safety:** The laboratory must meet Biosafety Level 2 Criteria as described in **Biosafety in Microbiological and Biomedical Laboratories,** 3rd Ed., HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, May, 1993. **Immunocompromised individuals must not work in or be admitted to this area**.

5. **QA/QC Procedures:** A formal QA document must be prepared and should follow the guidelines for a laboratory QA Plan, p. 7 in the **Manual for the Certification of Laboratories Analyzing Drinking Water**, 1990, U. S. Environmental Protection Agency Publication No. EPA/570/9-90/008, 3rd Ed., Washington, D.C., and Section II of this Manual. Laboratories must have a written QA program that applies QC practices necessary to minimize errors in laboratory operations that are attributable to personnel, equipment, supplies, processing procedures, or analytical methods. These include records of routine monitoring of equipment and instrumentation performance. Records of all QC checks must be available to the U.S. EPA for inspection. The procedures for the preparation of reagents and performance of the method must be followed exactly as written in the U.S. EPA ICR protozoan method. Reagents must be stored no longer than the designated shelf life.

6. **Record-Keeping And Data Reporting:** A record system must be in use for tracking the samples from sample collection through log-in, analyses and data reporting.

INFORMATION COLLECTION RULE

APPLICATION FOR APPROVAL OF PROTOZOAN LABS AND ANALYSTS [2]

Laboratory: _____

Address: _____

City: _____ **State:** _____ **Zip:** _____

Contact Person: _____

Title: _____

Telephone: ( ) _____ **Fax:** ( ) _____

Type of Laboratory: **Commercial** ____ **Utility** ____ **State** ____ **Academic** ____

Other (describe) _____

Principal Customers: **Environmental** ____ **Clinical** ____ **Other** _____

Type of Protozoa Analyses: ***Giardia*** ____ ***Cryptosporidium*** ____ ***Entamoeba*** ____

Other (describe) _____

PERSONNEL QUALIFICATIONS

Name, education, protozoan analysis experience and field in which acquired (water, wastewater, clinical, etc.)

Principal Analyst/Supervisor: _____

Education [University/Degree(s)]: _____

Experience: _____

_____

_____

_____

[2]Where additional pages are required, clearly mark them using the same headings as in this application form.

Analyst #1: _____

Education: _____

Experience: _____

Analyst #2: _____

Education: _____

Experience: _____

Analyst #3: _____

Education: _____

Experience: _____

Technician #1: _____

Education: _____

Experience: _____

Technician #2: _____

Education: _____

Experience: _____

Technician #3: _____

Education: _____

Experience: _____

ON-SITE LABORATORY EQUIPMENT AND INSTRUMENTATION

ITEM	On Order	Number	TYPE/MODEL
Autoclave			
Refrigerator			
Freezer			
pH Meter			
Analytical Balance			
Top-loader Balance			
Membrane Filtration Equipment (for epifluorescent staining)			
Hydrometer Set			
Reagent Grade Water Supply			
Slide Warmer			
Incubator			
Centrifuge			
Centrifuge Rotors			
Other(s) (describe)			
Place a "✓" in the "On Order" column next to items that are on order.			

MICROSCOPE CAPABILITY

Vendor Name: _____ **Model:** _____

Optical Capability:

Epifluorescence **Yes** _____ **No** _____

DIC **Yes** _____ **No** _____

Hoffman Modulation **Yes** _____ **No** _____

Mercury Lamp _____ **watt bulb**

FITC Cube Specs. _____ **nm exciter filter;**

_____ **nm beam splitting dichroic mirror; or**

_____ **nm barrier or suppression filter**

Objective Power	Type (Achromate, Neofluor, oil, etc.)	Numerical Aperture	Used with (Epifluor, D.I.C., etc.)

CURRENT LABORATORY PROGRAMS

Protozoan Method(s)	Number of Analyses Per Year Per Method

Sample Types (Matrices Tested): _____

_____

_____

Documented Laboratory QA Plan: **Yes____** **No____**

Laboratory is in compliance with state and local ordinances and laws for handling and disposal of pathogenic agents:

Yes____ **No____**

Comments: _____

_____

_____

Estimated number of water samples that can be analyzed for protozoa/month using the ICR method: _____

_____

_____

The above application information is complete and accurate to the best of my knowledge.

_____

Laboratory Manager or Designee

Submit Application to:

ICR Laboratory Coordinator
U. S. Environmental Protection Agency
Office of Ground Water and Drinking Water
Technical Support Division
26 West Martin Luther King Drive
Cincinnati, OH 45268

APPENDIX C. CHECKLIST FOR LABORATORY APPROVAL FOR *GIARDIA* AND *CRYPTOSPORIDIUM*

ICR Protozoan Laboratory Checklist			
Laboratory:			
Address:			
City:		**State:**	**Zip:**
Type of Laboratory (Check):			
Commercial:	**Utility:**	**State:**	**Academic:**
Other (Describe):			
Principal Customers: (Check)	**Environmental:** **Clinical:** **Other (Describe):**		
Type of Protozoan Analyses:	***Giardia:***	***Cryptosporidium:***	***Entamoeba:***
(Check each)	**Other (describe):**		
Laboratory Contact Person:			
Title:			
Telephone:		**Fax:**	
Principal Analyst/Supervisor Name:			
Analyst Name:			
Name of Person Being Evaluated:			
Laboratory Evaluated by:			**Date:**

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Are the personnel listed on the ICR approval application still with the laboratory?		
Are there any personnel in the laboratory not listed on the ICR approval application?		
Is the documentation available showing that the principal analyst/supervisor has analyzed 100 water and/or wastewater samples for *Giardia* and/or *Cryptosporidium*?		
Is the documentation available showing that the analyst has analyzed 50 water and/or wastewater samples for *Giardia* and/or *Cryptosporidium*?		
Is the laboratory well lighted (approximately 100 foot-candles of light intensity on work surfaces)?		
Are 6-10 ft of bench space available per analyst?		
Are the bench tops made of a smooth, impervious surface?		
Is the laboratory floor space sufficient for the stationary equipment?		
Is glassware washing equipment available?		
Is the laboratory neatly organized with unused equipment and supplies being stored (free of clutter)?		
Are high standards of cleanliness and prompt disposal of waste materials exhibited?		
Is the laboratory equipped with ultraviolet lights and under negative air pressure?		
Does the laboratory have a reagent grade water system?		
Does the laboratory have an autoclave?		
Does the laboratory have a refrigerator (4°C) with a -20°C freezer compartment?		
Does the laboratory have a pH meter associated with two or three calibration buffers?		
Does the laboratory have either an incubator or slide warming table calibrated to 37 ± 3°C?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Does the laboratory have either a top loader or pan balance associated with calibration weights?		
Does the laboratory have a properly maintained and adjusted stomacher?		
Does the laboratory have a Hoefer filtration manifold, model FH 255V?		
Are the well weights for the Hoefer manifold well maintained?		
Are the microscope slides the appropriate size?		
Is the laboratory using clear nail polish to seal the coverslips to the slides?		
Are the cover slips 25 mm^2 and No. 1½?		
Does the laboratory have a hydrometer set covering the range 1.0-2.0?		
Does the laboratory have an epifluorescent microscope equipped with either Hoffman modulation or differential interference contrast optics?		
Is the microscope easily changed from epifluorescent optics to either Hoffman modulation or differential interference contrast optics and vice versa?		
Does the laboratory have a 20X scanning objective with a numerical aperture of 0.6 on the microscope?		
Is the microscope equipped with an ocular micrometer or some other measuring device?		
Has the ocular micrometer been calibrated in conjunction with the 20X and the 100X objectives?		
Is a table of objective calibrations near the microscope?		
Does the laboratory have a stage micrometer?		
Does the laboratory have a 100X objective with a numerical aperture of 1.3 on the microscope?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Is the epifluorescent portion of the microscope equipped with an appropriate excitation and band pass filters for examining fluorescein isothiocyanate-labeled specimens (exciter filter: 450-490 nm; dichroic beamsplitting mirror 510 nm; barrier or suppression filter: 515-520 nm)?		
Is the mercury bulb in the epifluorescent lamp house either a 50 or a 100 watt bulb?		
Does the laboratory keep a log or have an hour totalizer on the transformer of the number of hours on the mercury bulb?		
Has the mercury bulb been used longer than 100 h in the case of 50 watt bulb or longer than 200 h in the case of a 100 watt bulb?		
Can the principal analyst/supervisor establish Köhler illumination on the microscope?		
Can the analyst establish Köhler illumination on the microscope?		
Can the principal analyst/supervisor focus both microscope eyepieces?		
Can the analyst focus both microscope eyepieces?		
Did the principal analyst/supervisor adjust the interpupillary distance?		
Did the analyst adjust the interpupillary distance?		
Does the laboratory have a large capacity centrifuge?		
Does the laboratory have a swinging bucket rotor capable of spinning 250 ml capacity or greater screw-cap conical bottles?		
Does the laboratory have a swinging bucket rotor capable of spinning 50 ml capacity conical screw-cap tubes?		
Does the laboratory have a formal QA laboratory plan prepared and ready for examination?		
Does the laboratory have records of all QC checks available for inspection?		
Does the laboratory have an adequate record system for tracking samples from collection through log-in, analysis and data reporting?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Is a positive and a negative **Quality Control** filter run with each week's batch of filters being analyzed?		
Is the laboratory using Commercial filters with Commercial LT-10 filter holders or Filterite filters with Filterite filter holders?		
Are the sampling filters 10 in (25.4 cm) long and 1 μm in nominal porosity?		
Is the sampling apparatus configured appropriately for raw water sampling?		
Is the sampling apparatus configured appropriately for finished water sampling?		
Is the sampling apparatus cleaned well before reshipment and/or use?		
Does the laboratory have a checklist or set of sampling instructions which are used, when sampling is done by someone other than laboratory personnel?		
Are reagents well labelled with preparation dates and who prepared the reagent?		
Does the laboratory have formulation or recipe cards for the preparation of 2.0% sodium thiosulfate, 10% neutral buffered formalin, phosphate buffered saline, 1% sodium dodecyl sulfate solution, 1% Tween 80 solution, elution solution, 2.5 M sucrose solution, Percoll-sucrose solution, the ethanol/glycerin dehydration series, DABCO-glycerin mounting medium, and 1% bovine serum albumin?		
Is the laboratory using Ensys's hydrofluor-combo kit for staining *Giardia* cysts and *Cryptosporidium* oocysts?		
Is the Ensys hydrofluor-combo kit still within the expiration time set by the manufacturer?		
Is the Percoll-sucrose solution used within a week of preparation?		
Is the elution solution used within a week of preparation?		
Is the DABCO-glycerin mounting medium discarded six months after preparation?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Is the 1% bovine serum albumin discarded six months after preparation?		
Are disposable cutting tools used to cut the sampling filter down to the core?		
Are the disposable cutting tool blades reused?		
Is the sampling filter in either a glass or stainless steel pan of the appropriate size, while it is being cut to the core?		
Are the filter fibers divided appropriately before hand washing?		
Is the total hand washing time a minimum of 30 min?		
Is stomacher washing done in two five minute intervals with redistribution of the filter fibers between the intervals?		
Is the right amount of 10% neutral buffered formalin added to the concentrated particulates at the appropriate time?		
Are the concentrated particulates diluted appropriately before the Percoll-sucrose flotation?		
Is the Percoll-sucrose gradient prepared correctly in a clear conical centrifuge tube?		
Is a centrifugation nomograph for determining relative centrifugal force (gravities) located close to the centrifuge(s)?		
Is the Percoll-sucrose gradient centrifuged correctly with slow acceleration and deceleration?		
Is the Percoll-sucrose gradient interface harvested appropriately after centrifugation?		
Is the final volume of the interface 5 ml, when harvesting is complete?		
Are 5-mm diameter 12-well red teflon heavy coated slides used to determine the correct sample volume per filter in the IFA staining procedure?		
Is the sample volume per filter in the IFA staining procedure done correctly?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Are support and Sartorius membranes handled with blunt end forceps initially?		
Are the support and Sartorius membranes properly hydrated before application to the manifold?		
Is the Hoefer manifold properly configured and adjusted before the addition of the support and Sartorius membranes?		
Do the Sartorius membrane filters the laboratory is using have a porosity between 0.2 and 1.2 μm?		
Is a positive and a negative **IFA Control** using a Sartorius filter run with each run of the manifold?		
Are the Hoefer manifold wells labelled well during the staining procedure?		
Does the sample application to the membranes on the manifold include rinses of the wells and membranes with 1% bovine serum albumin before and after application?		
Is the primary antibody diluted correctly with 1X phosphate buffered saline and goat serum?		
Is the right amount of primary antibody applied per membrane, and is it incubated for the correct amount of time?		
Is the primary antibody rinsed away correctly before the application of the secondary antibody?		
Is the secondary antibody diluted correctly?		
Is the right amount of secondary antibody applied per membrane, and is it incubated for the correct amount of time?		
Are the Hoefer manifold well weights covered with aluminum foil during the secondary antibody incubation?		
Is the secondary antibody rinsed away correctly after the incubation period?		
Is the alcohol dehydration step done correctly?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Are the glass slides that are to receive the membranes from the manifold labelled in advance?		
Have the labelled glass slides been prewarmed for 20-30 min with 75 μL of 2% DABCO-glycerin before the application of the membrane?		
Is a fresh, clean pair of forceps used to transfer each membrane from the Hoefer manifold to its respective glass slide?		
Is care exercised to insure that the Sartorius membranes are applied top side up to the slide?		
Are the membranes allowed to clear before application of the cover slip?		
Are the membranes flattened correctly, before sealing the cover slip?		
Are all the edges of the cover slip sealed well with clear nail polish?		
Is sample processing data being recorded as the method is being performed?		
Are the finished slides stored in an appropriate "dry-box"?		
Is the dry-box of slides allowed to reach room temperature before being opened?		
Is the microscope aligned and adjusted before the analysts starts scanning and reading slides?		
Is the scanning of the slides done appropriately, with the entire coverslip being scanned rather than just the membrane?		
Are measurements done with the 100X objective?		
Is the room in which the microscope is located darkened while the microscope is being used?		
Are the positive and negative control slides examined as prescribed in the method, including the complete examination of 3 *Giardia* cysts and 3 *Cryptosporidium* oocysts?		
Can the microscopist who is reading the sample slides easily change the optics from epifluorescence to Hoffman modulation or differential interference contrast optics?		

ICR Protozoan Laboratory Checklist		
Question	**Answer**	
	Yes	**No**
Are confirmations of internal structures within *Giardia* cysts and *Cryptosporidium* oocysts being confirmed by a principal analyst/supervisor.		
Is the microscopic data being entered onto the *Giardia* and *Cryptosporidium* report forms appropriately?		
Are the results from each sample being calculated on the provided computer spreadsheet?		
Are the computer spreadsheet files backed up on more than one disk, to insure data are not lost in the eventuality of some hardware failure?		
Are the Hoefer manifold and the stainless steel wells cleaned as prescribed in the method?		
Are the forceps used during the IFA staining cleaned well between uses?		
Is all glassware and plasticware washed well and stored appropriately between uses?		

ICR Protozoan Laboratory Checklist
Comments:

APPENDIX D. CHECKLIST FOR LABORATORY APPROVAL FOR TOTAL CULTURABLE VIRUS

SECTION I - LABORATORY-SPECIFIC INFORMATION

ICR Virus Laboratory Checklist			
Laboratory:			
Address:			
City:		**State:**	**Zip:**
Type of Laboratory (Check):			
Commercial:	**University:**	**Utility:**	**State:**
Other (Describe):			
Principal Customers: (Check)	**Environmental:** **Clinical:** **Other (Describe):**		
Laboratory Contact Person:			
Title:			
Telephone:		**Fax:**	
Laboratory Evaluated by:			**Date:**

1. Qualifications of Laboratory Personnel

Name	Position/Title	ICR Position	To Be Evaluated (Y/N)	Time in Present Position	Academic Training/ Degree	Job Training/ Experience/ Area

Codes for Marking Checklist

S - Satisfactory **U - Unsatisfactory** **NA- Not Applicable**

Item to be evaluated			Evaluation
2.	**Laboratory Facilities**		
2.1	Laboratory rooms are clean, and temperature and humidity controlled		
2.2	Lighting at bench top is adequate		
2.3	Bench tops have smooth, impervious surfaces		
2.4	Working space per analyst is adequate		
2.5	Storage space is adequate		
2.6	Work is separated by room or by microbiological hoods		
3.	**Laboratory Safety**		
3.1	Laboratory meets and follows "laboratory biosafety level 2 guidelines"		
3.2	Access to laboratory is limited		
3.3	Lab coats are used in the laboratory		
3.4	Mechanical pipetting devices are used		
3.5	Food is not stored or consumed in the laboratory		
3.6	Appropriate biohazard signs are placed on laboratory access doors		
3.7	A written biosafety manual is followed and available for inspection		
3.8	Laboratory personnel are adequately trained		
3.9	Laboratory has provision for disposal of microbiological wastes		
4.	**Laboratory Equipment and Supplies**		
4.1	***Laboratory pH Meter***		
	Manufacturer	Model	
	4.1.1 Accuracy ± 0.1 units; scale graduations, 0.1 units		
	4.1.2 pH buffer solution aliquots are used only once		
	4.1.3 Electrodes are maintained according to manufacturer's recommendations		

	Item to be evaluated		Evaluation
	4.1.4	Commercial buffer solutions are dated when received and discarded before expiration date	
QC	**4.1.5**	A record of pH measurements and calibrations used is maintained	
4.2	***Light Microscope***		
	Manufacturer	Model	
	4.2.1	Microscope is equipped with lenses to provide about 40X - 100X total magnification	
	4.2.2	Optical clarity is good	
4.3	***Inverted Light Microscope***		
	Manufacturer	Model	
	4.3.1	Microscope is equipped with lenses to provide about 40X - 100X total magnification	
	4.3.2	Optical clarity is good	
4.4	***Microbiological Hood***		
	Manufacturer	Model	
	4.4.1	Hood is at least a class II biological safety cabinet	
	4.4.2	Hood is certified on an annual basis	
4.5	***Temperature Monitoring***		
	4.5.1	Glass/mercury, dial thermometers or continuous recording devices are used with appropriate equipment. Units are graduated in no more than 0.5°C increments. Mercury columns are not separated	
QC	**4.5.2**	Calibration of glass/mercury thermometers is checked annually and dial thermometers quarterly at the temperature used against a reference NIST thermometer or one meeting the requirements of NIST Monograph SP 250-23	
QC	**4.5.3**	Correction data are available for reference thermometers	
QC	**4.5.4**	Continuous recording devices are recalibrated annually	

	Item to be evaluated		Evaluation
4.6	***Incubator***		
	Manufacturer	Model	
	4.6.1 An internal temperature of 36.5 ± 1°C is maintained		
	4.6.2 A temperature monitoring device is placed on a shelf near area of use. The bulb or probe of the temperature monitoring device is in liquid		
QC	**4.6.3** Temperature is recorded at least once per day for each workday in use		
4.7	***Refrigerator***		
	Manufacturer	Model	
	4.7.1 An internal temperature of 1° to 5°C is maintained		
	4.7.2 A temperature monitoring device is placed on a shelf near area of use. The bulb or probe of the temperature monitoring device is in liquid		
QC	**4.7.3** Temperature is recorded at least once per day for each workday in use		
4.8	***Freezer, -20 ℃***		
	Manufacturer	Model	
	4.8.1 An internal temperature of -20° ± 5°C is maintained		
	4.8.2 A temperature monitoring device is placed on a shelf near area of use.		
QC	**4.8.3** Temperature is recorded at least once per day for each workday in use		
4.9	***Freezer, -70 ℃***		
	Manufacturer	Model	
	4.9.1 An internal temperature of -70° ± 3°C or lower is maintained		
	4.9.2 A temperature monitoring device is placed on a shelf near area of use		

Item to be evaluated			Evaluation
QC	**4.9.3**	Temperature is recorded at least once per day for each workday in use	
4.10	***Refrigerated Centrifuge***		
	Manufacturer	Model	
	4.10.1	Operates at a centrifugal force of at least 4,000 ×*g*	
	4.10.2	Holds at 4°C during centrifugation run	
	4.10.3	Appropriate rotor holds 100 - 1000 ml bottles	
QC	**4.10.4**	A log recording rotor serial number, run speed and time, run temperature and operator's initials is kept for each centrifugation run	
4.11	***Balance***		
	Manufacturer	Model	
QC	**4.11.1**	Balance is calibrated monthly	
QC	**4.11.2**	Correction data are available for S/S-1 calibration weights	
	4.11.3	An annual service contract or internal maintenance protocol is maintained	
4.12	***Autoclave***		
	Manufacturer	Model	
	4.12.1	Unit is equipped with a temperature gauge with sensor on exhaust	
	4.12.2	Unit depressurizes slowly so that media do not boil over	
	4.12.3	Unit’s automatic timing mechanism is adequate	
	4.12.4	A service contract or internal maintenance protocol is maintained	
	4.12.5	A maximum temperature-registering thermometer or heat-sensitive tape is used with each cycle	
QC	**4.12.6**	Spore strips or ampoules are used on a monthly basis	
QC	**4.12.7**	Date, contents, sterilization time and temperature are re-corded for each cycle	
4.13	***Hot Air Oven (if used)***		
	Manufacturer	Model	

	Item to be evaluated		Evaluation
	4.13.1 Hot air oven maintains temperature of 170 - 180°C for at least 2 h		
	4.13.2 Bulb or probe of temperature monitoring device is placed in sand during use. Thermometer graduated in no more than 10°C increments		
QC	**4.13.3** Date, sterilization time and temperature are recorded for each cycle		
4.14	***Pump***		
	Manufacturer	Model	
	Pump is self-priming		
4.15	***Polypropylene Container***		
	Manufacturer/Source	Model/Cat. No.	
	Container holds 40 L; contents can be mixed without spilling		
4.16	***Positive Pressure Source*** *(record for source used)*		
	Compressed air		
	Compressed nitrogen		
	Laboratory air source		
	Manufacturer	Model	
	Peristaltic pump		
	Manufacturer	Model	
4.17	***Magnetic Stirrer***		
	Manufacturer	Model	
4.18	***Source for Reagent Grade Water***		
	Type/Manufacturer	Model/Cat. #	
	4.18.1 Still or deionization unit is maintained according to manufacturer's instructions		
	4.18.2 Reagent grade water is used to prepare all media and reagents		
QC	**4.18.3** The conductivity is tested with each use. Conductivity is >0.5 megohms-cm at 25°C		

Item to be evaluated	Evaluation
5. General Laboratory Practices	
5.1 ***Analytical Media***	
5.1.1 General	
5.1.1.1 Commercial media and chemicals are dated upon receipt. Only analytical reagent or ACS grade chemicals are used for preparation of media	
5.1.1.2 Commercial dehydrated or liquid media are used for propagation of tissue culture cells. Dehydrated media are prepared and stored as recommended by manufacturers.	
5.1.1.3 Commercial media and chemicals are discarded by manufacturers' expiration dates. Laboratory prepared media are discarded by the expiration dates indicated in the Virus Monitoring Protocol	
5.1.1.4 Each lot of medium is checked for sterility before use	
QC 5.1.1.5 Lot numbers of commercial media and chemicals are recorded. Date of preparation, type of medium, lot number, sterilization procedure, pH and technician's initials are recorded for laboratory prepared media	
5.1.2 Thiosulfate (2%)	
Solutions are stored at or below room temperature and discarded after six months	
5.1.3 Hydrochloric acid	
5.1.3.1 Solutions are prepared at least 24 h prior to use in sampling or virus assays	
5.1.3.2 Solutions are stored at or below room temperature and discarded after six months	
5.1.4 Sodium Hydroxide	
5.1.4.1 Solutions are prepared at least 24 h prior to use in virus assays	
5.1.4.2 Solutions are stored in polypropylene containers at room temperature and discarded after 3 months	
5.1.5 Beef Extract (1.5%)	
5.1.5.1 Final pH is 9.5	

Item to be evaluated	Evaluation
5.1.5.2 Solution is stored at 4°C and discarded after one week or at -20°C and discarded after 18 months	
5.1.6 Sodium Phosphate	
5.1.6.1 Final pH is between 9.0 and 9.5	
5.1.6.2 Solutions are stored at or below room temperature and discarded after six months	
5.1.7 Washing Solution	
5.1.7.1 Salt solution is cooled to room temperature before addition of serum	
5.1.7.2 Solutions are stored at 4°C and discarded after 3 months or at -20°C and discarded after 18 months	
5.1.8 Chlorine	
5.1.8.1 Final pH is between 6 and 7	
5.1.8.2 Solutions are stored at or below room temperature and discarded after one month	
5.1.9 Iodine	
Solutions are stored at room temperature and discarded after six months	
5.2 ***Sterilization and Disinfection***	
5.2.1 Autoclavable glassware, plasticware and equipment are autoclaved at 121°C for 1 h or, if appropriate, sterilized by dry heat at 170°C for at least 1 h	
5.2.2 Non-autoclavable supplies are disinfected with 0.1% chlorine (pH 6-7) for 30 min or in a gas sterilizer according to the manufacturer's recommendations	
5.2.3 Contaminated materials are autoclaved at 121°C for at least 1 h	
5.2.4 Adequate glassware washing facilities are available for reusable lab ware	
5.2.5 Surfaces are disinfected before and after use and after spills	
7. Quality Assurance	
A written QA plan is followed and available for inspection	

SECTION II - ANALYST-SPECIFIC INFORMATION (To be filled out for each principal analyst/analyst/technician seeking approval for ICR virus analysis):

Name of Analyst/Technician:		
Item to be evaluated		**Evaluation**
6. Analytical Methodology		
6.1 *General*		
	Only the virus analytical method dated July, 1995, is used for site visit evaluation	
6.2 *QC Samples*		
	A polypropylene container and pump are used to pump a negative QC sample through a 1MDS filter in a standard sampling apparatus. All components of the system are sterile	
6.3 *Filter Elution*		
	6.3.1 Residual water is blown out from the cartridge housing before addition of beef extract	
	6.3.2 1MDS filters are slowly eluted with 1.5% beef extract twice. The flow of beef extract is interrupted for 1 min during each pass to enhance elution	
	6.3.3 An air filter is used with a positive pressure lab air source	
6.4 *Organic Flocculation*		
QC	**6.4.1** The pH meter is standardized at pH 4 and 7	
	6.4.2 The pH electrode is disinfected before and after use	
	6.4.3 The pH of the eluate is adjusted slowly to 3.5 ± 0.1 with 1 M HCl with stirring at a speed sufficient to develop a vortex	
	6.4.4 The eluate is stirred for 30 min after pH adjustment	
	6.4.5 The pH adjusted eluate is centrifuged at 2,500 ×*g* for 15 min at 4°C.	
	6.4.6 Supernatant from centrifuge run is properly discarded	
	6.4.7 Precipitate from centrifuge run is dissolved in 30 ml of 0.15 M sodium phosphate.	
QC	**6.4.8** The pH meter is standardized at pH 7.0 and 10.0	
	6.4.9 The pH electrode is disinfected before and after use	

Name of Analyst/Technician:		
	Item to be evaluated	**Evaluation**
	6.4.10 The pH of the dissolved precipitate is checked and readjusted to 9.0-9.5, if necessary	
	6.4.11 The dissolved precipitate is centrifuged at 4,000-10,000 ×*g* for 10 min at 4°C	
	6.4.12 The supernatant from the 4,000-10,000 ×*g* run is saved and the precipitate properly discarded	
	6.4.13 The pH of the supernatant is adjusted to 7.0-7.5 with 1 M HCl	
	6.4.14 The supernatant is treated to remove or reduce microbial contamination. Sterilizing filters are pretreated before use with beef extract	
	6.4.15 The final volume is recorded after treatment	
	6.4.16 The treated supernatant is divided into subsamples.	
6.5 ***Total Culturable Virus Assay***		
QC	**6.5.1** Passage 117 to 250 BGM cells from the U.S. EPA are being cultured for ICR virus assays	
	6.5.2 Cultures are used 3-6 days after passage. Cultures are washed prior to inoculation with serum free medium	
	6.5.3 At least 10 replicate cultures per subsample or subsample dilution are inoculated with a proper inoculation volume	
	6.5.4 Inoculation volume does not exceed 0.04 ml/cm^2	
	6.5.5 An adsorption period of 80-120 min is used. Adsorption occurs at 22 to 36.5 ± 1°C	
	6.5.6 Liquid maintenance medium is added and cultures are incubated at 36.5 ± 1°C	
	6.5.7 A 2nd passage is performed using 10% of the medium from the 1st passage. Samples positive in the 1st passage are filtered prior to passage	
	6.5.8 Analyst demonstrates ability to perform MPN calculations	
	6.5.9 A positive and negative control is run with each sample	

DESCRIPTION OF CHECKLIST FOR LAB APPROVAL FOR VIRUS ANALYSIS

Note: Written records must be retained for five years for quality control items designated as "QC".

1. Personnel

1.1 *Principal Analyst/Supervisor*
The principal analyst/supervisor is a qualified microbiologist with experience with environmental virology. The principal analyst/supervisor oversees all analyses of samples for viruses.

1.1.1 Academic Training: Minimum of a bachelor's degree in the life sciences.

1.1.2 Job Training: Minimum of three years experience in cell culture and animal virus analyses.

1.2 *Analyst*
The analyst performs at the bench level with minimal supervision and is involved in all aspects of the analysis, including sample collection, filter extraction, sample processing and assay.

1.2.1 Academic Training: Minimum of two years of full time college with a major in life science.

1.2.2 Job Training: Minimum of six months of full-time bench experience in cell culture and animal virus analyses.

1.3 *Technician*
The technician extracts the filter and processes samples, but does not perform tissue culture work.

1.3.1 Academic Training: No requirements.

1.3.2 Job Training: Three months experience in filter extraction of virus samples and sample processing.

2. Laboratory Facilities

2.1 Laboratory facilities are temperature and humidity controlled. Laboratories are clean; a pest control program is in place, if appropriate.

2.2 Work surfaces have adequate lighting (minimum of 100 foot-candles).

2.3 Laboratory bench tops have smooth, impervious surfaces.

2.4 There is at least six to ten linear feet of usable bench space per analyst with a minimum of 36-38 inches of depth.

2.5 There is sufficient laboratory space for storage of media, glassware and equipment.

2.6 Filter extraction/sample processing is performed in a separate laboratory room from cell culture and virus work. Cell culture and virus work are performed in separate rooms or in separate microbiological hoods. A program is in place to ensure that no cross-contamination occurs if the latter is used.

3. Laboratory Safety

3.1 The laboratory meets and follows laboratory biosafety level 2 guidelines.

3.2 Laboratories have limited access.

3.3 Lab coats are worn while working in laboratories.

3.4 Mouth pipetting is not allowed in the laboratory.

3.5 Food and drinks are not stored or consumed in the laboratory.

3.6 Biohazard signs identifying biohazards are placed on the laboratory access doors.

3.7 A written biosafety manual is followed and available for inspection.

3.8 Laboratory personnel have been given laboratory safety training.

3.9 The laboratory is in compliance with all applicable judicial ordinances and laws for virus work and biological waste disposal.

4. Laboratory Equipment and Supplies

4.1 pH Meters

4.1.1 The accuracy and scale graduations of a laboratory pH meter are within ±0.1 pH units. The accuracy and scale graduations of a portable pH meter for use with water sampling are within ±0.2 pH units.

4.1.2 pH buffer aliquots are used only once.

4.1.3 Electrodes are maintained according to the manufacturer's recommendations.

QC **4.1.4** Commercial buffer solution containers are dated upon receipt and when opened. Solutions are discarded before the expiration date.

4.2 ***Light Microscope***

4.2.1 The microscope is equipped with lenses to provide about 40X to 100X total magnification.

4.2.2 Optical clarity is sufficient to accurately count cells in a hemocytometer.

4.3 ***Inverted Light Microscope***

4.3.1 The microscope is equipped with lenses to provide about 40X to 100X total magnification.

4.3.2 Optical clarity is sufficient to accurately demonstrate CPE.

4.4 ***Microbiological hood*** (if separate work areas are not available)

4.4.1 Hood is at least a class II biological safety cabinet.

QC **4.4.2** Hood is certified to be in proper operating condition on at least an annual basis.

4.5 ***Temperature Monitoring***

4.5.1 Glass/mercury, dial thermometers or continuous recording devices are used to monitor equipment. Units are graduated in 0.5°C increments or less. Mercury columns in glass thermometers are not separated.

QC **4.5.2** The calibration at the temperature used of each glass/mercury thermometer is checked annually against a reference National Institute of Standards and Technology (formerly National Bureau of Standards) (NBS) thermometer or one that meets the requirements of NIST Monograph SP 250-23. The calibration of each in-use dial thermometer is checked quarterly.

QC **4.5.3** Correction data are available for all reference thermometers used for calibration.

QC **4.5.4** Continuous recording devices are recalibrated annually using the reference thermometer described in QC 4.5.2.

4.6 ***Incubator***

4.6.1 The incubator maintains an internal temperature of 36.5 ± 1°C.

4.6.2 A temperature monitoring device is placed on a shelf near area of use. The bulb or probe of the temperature monitoring device is in liquid.

QC **4.6.3** The temperature is recorded at least once per day for each workday in use.

4.7 ***Refrigerator***

4.7.1 The refrigerator maintains a temperature of 1° to 5°C.

4.7.2 A calibrated temperature monitoring device is placed on a shelf near the area of use. The thermometer bulb or probe is immersed in liquid.

QC **4.7.3** The temperature is recorded at least once per day for each workday in use.

4.8 **Freezer, -20°C**

4.8.1 The freezer maintains a temperature of -20 ± 5°C. The freezer may be a compartment associated with 4.6.

4.8.2 A calibrated temperature monitoring device is placed on a shelf near the area of use.

QC **4.8.3** The temperature is recorded at least once per day for each workday in use.

4.9 ***Freezer, -70°C***

4.9.1 The freezer maintains a temperature of -70 ± 3°C or lower.

4.9.2 A calibrated temperature monitoring device is placed on a shelf near the area of use.

QC **4.9.3** The temperature is recorded continuously during periods of use or at least once per day for each workday in use.

4.10 ***Refrigerated Centrifuge***

4.10.1 The centrifuge can be operated at a centrifugal force of at least 4,000 ×*g*.

4.10.2 Centrifuge maintains an internal temperature of 4°C during run.

4.10.3 A rotor is available which is capable of 4,000 ×*g* while holding centrifuge bottles of 100 - 1000 ml.

QC **4.10.4** A log recording rotor serial number, run speed, time of centrifugation, temperature of operation and operator is kept for each centrifuge run.

4.11 ***Balance***

QC **4.11.1** The balance is calibrated monthly using Class S or S-1 reference weights (minimum of three traceable weights which bracket laboratory weighing needs) or weights traceable to Class S or S-1 weights.

QC **4.11.2** Correction data are available for the S or S-1 calibration weights.

4.11.3 A service contract or internal maintenance protocol is established and records are maintained.

4.12 ***Autoclave***

4.12.1 The autoclave has a temperature gauge with a sensor on the exhaust, a pressure gauge and an operational safety valve.

4.12.2 Autoclave depressurizes slowly to ensure that media do not boil over.

4.12.3 The autoclave's automatic timing mechanism is adequate. The autoclave maintains sterilization temperature during the sterilizing cycle and completes an entire liquid cycle within 45 min when a 12-15 min sterilization period is used.

4.12.4 A service contract or internal maintenance protocol is established and records are maintained.

4.12.5 A maximum temperature-registering thermometer or heat-sensitive tape is used with each autoclave cycle.

QC **4.12.6** Spore strips or ampules are used on a monthly basis.

QC **4.12.7** The date, contents, sterilization time and temperature is recorded for each cycle.

4.13 ***Hot Air Oven*** (If used for sterilizing dry glassware.)

4.13.1 The oven maintains a stable sterilization temperature of 170 - 180°C for at least two h.

4.13.2 A temperature monitoring device is used with the bulb or probe placed in sand during use. The monitoring device is graduated in no more than 10°C increments.

QC **4.13.3** The date, contents, sterilization time and temperature is recorded for each cycle.

4.14 ***Pump***

A self-priming pump is required for preparation of QC samples. It is recommended that the pump be capable of pumping at a rate of 3 gal/min at 30 PSI.

4.15 Polypropylene Container

The container holds at least 40 L. The contents can be mixed without spilling or splashing.

4.16 ***Positive Pressure Source***

An air or nitrogen source and pressure vessel or a peristaltic type pump is used for filter elution.

4.17 ***Magnetic Stirrer***

The magnetic stirrer is capable of maintaining a vortex during organic flocculation and pH adjustments.

4.18 ***Source for Reagent Grade Water***

4.18.1 Distillation and/or deionization units are maintained according to the manufacturer's instructions or water is purchased commercially.

4.18.2 Reagent grade water is used to prepare all media and reagents.

QC **4.18.3** The conductivity of the reagent grade water is tested with each use. The conductivity is >0.5 megohms-cm at 25°C.

5. General Laboratory Practices

5.1 ***Analytical Media***

5.1.1 General

5.1.1.1 Commercial media and chemicals are dated upon receipt and when first opened. Only analytical reagent or ACS grade chemicals are used for the preparation of media.

5.1.1.2 Use of commercial dehydrated or liquid media for propagation of tissue culture cells are recommended due to concern about quality control. Dehydrated media are prepared and stored as recommended by the manufacturers.

5.1.1.3 Commercial media and chemicals are discarded by manufacturers' expiration dates. Laboratory prepared media are discarded by the expiration dates indicated in the Virus Monitoring Protocol.

5.1.1.4 Each lot of medium is checked for sterility before use as described in the Virus Monitoring Protocol.

QC 5.1.1.5 The lot numbers of commercial media and chemicals are recorded. The date of preparation, type of medium, lot number, sterilization procedure, pH and technician's initials are recorded for media prepared in the laboratory.

5.1.2 Thiosulfate (2%)

5.1.2.1 A stock solution of 2% thiosulfate is prepared by dissolving 100 g of $Na_2S_2O_3$ in a total of 5000 ml of reagent grade water. The solution is autoclaved for 30 min at 121°C.

5.1.2.2 2% thiosulfate is stored at or below room temperature for up to six months.

5.1.3 Hydrochloric acid (HCl)

5.1.3.1 Solutions of 0.1, 1 and 5 M HCl are prepared by mixing 50, 100 or 50 ml of concentrated HCl with 4950, 900 or 50 ml of reagent grade water, respectively. Solutions of HCl are self-sterilizing and should be prepared at least 24 h prior to use.

5.1.3.2 Solutions of HCl are stored at or below room temperature for up to six months.

5.1.4 Sodium Hydroxide (NaOH)

5.1.4.1 Solutions of 1 M and 5 M NaOH are prepared by dissolving 4 or 20 g of NaOH in a final volume of 100 ml of reagent grade water, respectively. Solutions of NaOH are self-sterilizing and should be prepared at least 24 h prior to use.

5.1.4.2 Solutions of NaOH are stored in polypropylene containers at room temperature for up to three months.

5.1.5 Beef Extract, 1.5%

5.1.5.1 Buffered 1.5% beef extract is prepared by dissolving 30 g of beef extract V powder and 7.5 g of glycine (final glycine concentration = 0.05 M) in 1.9 L of reagent grade water. The pH is adjusted to 9.5 with 1 or 5 M NaOH and the final

volume is brought to 2 L with reagent grade water. The solution is autoclaved at 121°C for 15 min.

5.1.5.2 Solutions of 1.5% beef extract are stored for one week at 4°C or for up to 18 months at -20°C.

5.1.6 Sodium Phosphate, 0.15 M

5.1.6.1 A solution of 0.15 M sodium phosphate is prepared by dissolving 40.2 g of sodium phosphate ($Na_2HPO_4 \bullet 7H_2O$) in a final volume of 1000 ml of reagent grade water. The pH is checked to ensure that it is between 9.0 - 9.5 and adjusted with 1 M NaOH, if necessary. The solution is autoclaved at 121°C for 15 min.

5.1.6.2 Solutions of 0.15 M sodium phosphate are stored at or below room temperature for up to six months.

5.1.7 Washing Solution

5.1.7.1 Washing solution is prepared by dissolving 8.5 g of NaCl in a final volume of 980 ml of reagent grade water. The solution is autoclaved at 121°C for 15 min and cooled to room temperature. 20 ml of bovine serum is added and the solution is mixed thoroughly.

5.1.7.2 The wash solution is stored at 4°C for up to three months or at -20°C for up to 18 months.

5.1.8 Chlorine, 0.1%

5.1.8.1 A solution of 0.1% chlorine (HOCl) is prepared by adding 19 ml of household bleach to 900 ml of reagent grade water, adjusting the pH of the solution to 6-7 with 1 M HCl and bringing the final volume to 1 L with reagent grade water. Solutions of 0.1% chlorine are self-sterilizing.

5.1.8.2 Solutions of 0.1% chlorine are stored at or below room temperature for up to one month.

5.1.9 Iodine, 0.5%

5.1.9.1 A solution of 0.5% iodine is prepared by dissolving 5 g I_2 in 1000 ml of 70% ethanol. Solutions of 0.5% iodine are self-sterilizing.

5.1.9.2 Solutions of 0.5% iodine are stored at room temperature for up to six months.

5.2 *Sterilization and Disinfection*

5.2.1 Autoclavable glassware, plasticware and equipment are sterilized by autoclaving at 121°C for 1 h or, if appropriate, by dry heat at 170°C for at least one h.

5.2.2 Non-autoclavable supplies are disinfected with 0.1% chlorine (pH 6-7) for 30 min or in a gas sterilizer according to the manufacturer's instructions.

5.2.3 Contaminated materials are sterilized by autoclaving at 121°C for at least 1 h.

5.2.4 Adequate glassware washing facilities are available for washing re-usable glassware.

5.2.5 All surfaces are disinfected with 0.5% iodine or 0.1% chlorine, pH 6-7 before and after each use and after any spill or other contamination.

6. Analytical Methodology

6.1 *General*

Only the analytical methodology specified in the July, 1995, draft of the *Virus Monitoring Protocol for the Information Collection Rule* is used for lab and analyst approval.

6.2 *QC Samples*

QC Each analyst and technician must prepare and process a negative QC sample during the site visit (technicians will only be required to perform steps 6.3 to 6.4). A negative QC sample is prepared by pumping 40 L of reagent grade water placed in a sterile polypropylene container through a sterile standard sampling apparatus.

6.3 *Filter Elution*

6.3.1 Residual water is blown out from the cartridge housing.

6.3.2 Virus is eluted from the 1MDS filter by slowly passing 1000 ml of 1.5% beef extract (pH 9.5) through the filter twice. The flow of beef extract is interrupted for 1 min during each pass to enhance elution.

6.3.3 An air filter is used with a positive pressure lab air source.

6.4 ***Organic Flocculation***

QC **6.4.1** The pH meter is standardized at pH 4 and 7.

6.4.2 The pH electrode is disinfected before and after use.

6.4.3 The pH of the eluate is adjusted slowly to 3.5 ± 0.1 with 1 M HCl with stirring at a speed sufficient to develop a vortex.

6.4.4 The eluate is stirred for 30 min after pH adjustment.

6.4.5 The pH adjusted eluate is centrifuged at 2,500 ×*g* for 15 min at 4°C.

6.4.6 The supernatant is properly discarded after the centrifugation run.

6.4.7 The precipitate is dissolved in 30 ml of 0.15 M sodium phosphate.

QC **6.4.8** The pH meter is standardized at pH 7 and 10.

6.4.9 The pH electrode is disinfected before and after use.

6.4.10 The pH of the dissolved precipitate is readjusted to 9.0 - 9.5, if necessary.

6.4.11 The dissolved precipitate is centrifuged at 4,000 - 10,000 ×*g* for 10 min at 4°C.

6.4.12 The supernatant is removed and saved after the centrifugation run. The pellet is properly discarded.

6.4.13 The pH of the supernatant is adjusted to 7.0 - 7.5 with 1 M HCl.

6.4.14 The supernatant is treated to remove or reduce microbial contamination. Sterilizing filters are pretreated before use with beef extract.

6.4.15 The final volume is recorded after treatment.

6.4.16 The treated supernatant is divided into subsamples.

6.5 ***Total Culturable Virus Assay***

QC **6.5.1** Passage 117 to 250 BGM cell cultures obtained from the U.S. EPA are being cultured for ICR virus assays.

6.5.2 Cultures are used between three and six days after the most recent passage or the laboratory has demonstrated that the culture time used is as sensitive as cultures at three to six days. Cultures are washed prior to inoculation with serum-free medium.

6.5.3 At least ten replicate cultures per subsample or subsample dilution are inoculated with an inoculation volume equal to 1/20th the assay sample volume.

6.4.4 The inoculation volume does not exceed 0.04 ml/cm^2.

6.5.5 Virus is allowed to adsorb onto cells for 80 - 120 min at room temperature or at 36.5 ± 1°C.

6.5.6 Liquid maintenance medium is added and cultures are incubated at 36.5 ± 1°C.

6.5.7 A 2nd passage is performed using 10% of the medium from the 1st passage. Samples that were positive in the 1st passage are filtered before doing the 2nd passage.

6.5.8 The analyst demonstrates the ability to perform MPN calculations.

6.5.9 A positive and negative control is run with each sample.

7. Quality Assurance

The laboratory prepares and follows a written QA plan which is available for inspection during the site visit.